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Hyperglycaemia in infants with hypoxic ischaemic encephalopathy is associated with improved outcomes after therapeutic hypothermia: a post-hoc analysis of the CoolCap Study

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Short title: Glycaemic profile predicts hypothermia response in HIE
Abbreviations: aRR, adjusted risk ratio; ALT, alanine aminotransferase; ANOVA, analysis of variance; AST, aspartate aminotransferase; BP, blood pressure; CI, confidence interval; EEG, electroencephalogram; FiO_2, fraction of inspired oxygen; HCO_3, bicarbonate; HIE, hypoxic ischaemic encephalopathy; HUS, head ultrasound; IQR, inter quartile range; MAP, mean arterial pressure; NNT, number needed to treat; OR, odds ratio; $PaCO_2$, partial pressure of carbon dioxide; PaO_2, partial pressure of oxygen; PT, prothrombin time; PTT, partial thromboplastin time.
Key words: hypoglycaemia; hyperglycaemia; phenotype; neonatal; hypoxic ischaemic encephalopathy; CoolCap; unfavourable outcome.
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What is already known on this topic

- Hypoxic-ischaemic encephalopathy (HIE) is characterized by evolving neurologic dysfunction and frequently, systemic multi-organ injury.
- Hypoglycaemia and hyperglycaemia in infants with HIE are common and are associated with unfavourable outcomes.

What this study adds

- Early glycaemic profile in infants with moderate-to-severe HIE is associated with both the risk of multi-organ dysfunction and outcome of therapeutic hypothermia.
- In the CoolCap Study, only infants with early hyperglycaemia significantly benefited from therapeutic hypothermia.

ABSTRACT

Objective To investigate whether glycaemic profile is associated with multi-organ dysfunction and with response to hypothermia after perinatal hypoxic ischaemic encephalopathy (HIE).
Design Post hoc analysis of the CoolCap Study.
Setting 25 perinatal centres in UK, USA, and New Zealand during 1999–2002.
Patients 194/234 (83%) infants of ≥36 weeks' gestation with moderate-to-severe HIE enrolled in the CoolCap Study with documented plasma glucose levels and follow-up outcome.
Intervention Infants were randomised to head cooling for 72 h starting within 6 h of birth, or standard care. Plasma glucose levels were measured at pre-determined time intervals after randomisation.
Main outcome measure Unfavourable primary outcome was defined as death and/or severe neurodevelopmental disability at 18 months. Glycaemic profile (hypoglycaemia [≤40 mg/dL, ≤2.2 mmol/L], hyperglycaemia [>150 mg/dL, >8.3 mmol/L], and normoglycaemia) during 12 h after randomisation was investigated for association with multi-organ dysfunction, or risk reduction of primary outcome after hypothermia treatment.
Results Hypoglycaemia but not hyperglycaemia was associated with more deranged multi-organ function parameters (mean pH 7.23 [SD 0.16] vs 7.36 [0.13], p<0.001; aspartate transaminase 2101 [2450] vs 318 [516] IU/L, p=0.002; creatinine 1.95 [0.59] vs 1.26 [0.5] mg/dL, p<0.001) compared to normoglycaemia. After adjusting for Sarnat stage and 5-min Apgar score, only hyperglycaemic infants randomised to hypothermia had reduced risk of unfavourable outcome (adjusted risk ratio: 0.80, 95% CI 0.66 to 0.99), whereas hypoglycaemic and normoglycaemic infants did not.
Conclusions Early glycaemic profile in infants with moderate-to-severe HIE may help to identify risk of multi-organ dysfunction and response to therapeutic hypothermia.

INTRODUCTION

Perinatal hypoxic ischaemic encephalopathy (HIE) is characterized by evolving neurologic dysfunction and frequently, systemic multi-organ injury.[1, 2] Mild hypothermia induced within 6 h of birth for near term and term infants with moderate-to-severe HIE improves survival without disability, and the benefits persist to school age.[3-6] In the absence of a sentinel event, however, the timing, duration, and chronicity of fetal hypoxia-ischaemia remain uncertain. Universal application of therapeutic hypothermia, without consideration for the heterogeneous pathophysiologic mechanisms of injury, may be suboptimal, as many treated infants with moderate-to-severe HIE still die or survive with severe neurodevelopmental and cognitive impairment.[7] This emphasises the importance of investigating whether laboratory and clinical parameters can be identified early after birth, to predict the response to hypothermia treatment, and perhaps to guide the choice of individualised adjuvant neuroprotective therapies based on specific underlying pathophysiology among this heterogeneous population.

Glucose is the primary substrate for energy metabolism in the newborn brain, and deranged glucose homoeostasis may be a biomarker of, or contribute to, neuronal injury and adverse long-term outcomes.[8-12] At birth, the continuous transplacental supply of glucose ends; normal glucose homoeostasis then depends on intact multi-organ function and hormonal regulation.[13] Given the central role of glucose homoeostasis in cell metabolism, potentially early glycaemic status could contribute to the risk of multi-organ injury and recovery in HIE.[14]

We previously reported that early deranged glucose homoeostasis in infants with HIE was common, and that compared to normoglycaemia, both hypoglycaemia (≤40 mg/dL, ≤2.2 mmol/L) and hyperglycaemia (>150 mg/dL, >8.3 mmol/L) were associated with greater risk (adjusted odds ratio [aOR] 6.2 and 2.7, respectively) of unfavourable outcome at 18 months.[15, 16]

In the current study, we examined the hypotheses first, that early glycaemic perturbations (i.e., hypoglycaemia and hyperglycaemia) are biomarkers for the severity, chronicity, and timing of perinatal hypoxia-ischaemia and so would be associated with risk of multi-organ dysfunction, and second, that glycaemic status would be associated with benefit from therapeutic hypothermia. We reanalysed the CoolCap Study cohort using a different statistical approach, exploring the laboratory and clinical characteristics of infants by their early glycemic profile (hypoglycaemia, hyperglycaemia, and normoglycaemia within the first 12 h after randomization), to investigate for associations with multi-organ dysfunction. We then evaluated whether glycaemic status was associated with the outcome of therapeutic hypothermia for the primary outcome of death and/or severe disability at 18 months of age.

METHODS

Participants

The CoolCap Study was a multicentre randomised controlled trial of selective head cooling and mild systemic hypothermia for the treatment of perinatal moderate-to-severe HIE in 234 infants at ≥36 weeks' gestation enrolled between 1999 and 2002.[16] The institutional review board of each centre approved the protocol, and subjects were randomised to either head cooling for 72 h, starting within 6 h of birth, with rectal temperature maintained at 34.5±0.5°C, followed by rewarming over 4 h, or standard care at 37.0±0.5°C. The primary outcome was death or severe disability at 18 months (≥1 of the following: gross motor function classification system level 3–5, Bayley Scales of Infant Development II mental developmental index <70, and/or bilateral cortical visual impairment). In this post hoc analysis, subjects lost to follow-up (n=16), those with undocumented glucose values (n=4), infants missing Sarnat stage at randomisation (n=4), infants diagnosed with Sarnat stage 1 HIE at randomisation (n=8), or those who had both hypoglycaemia and hyperglycaemia during the first 12 h (n=8) were excluded.[17] Therefore, our analyses included 194 of the 234 (83%) infants enrolled in the original CoolCap Study (figure 1).

Data collected

Plasma glucose levels, collected at pre-specified time points (0, 4, 8, and 12 h after randomisation) were used to classify infants into three groups based on their glycaemic profile during the first 12 h after randomisation: (1) hypoglycaemia (≥1 glucose level ≤40 mg/dL, ≤2.2 mmol/L),[18] (2) hyperglycaemia (≥1 glucose level >150 mg/dL, >8.3 mmol/L),[11] and (3) normoglycaemia (all glucose levels >40 to ≤150 mg/dL, >2.2 to ≤8.3 mmol/L).[15] Demographic and perinatal data, postnatal clinical and laboratory parameters of multi-organ function (collected

at pre-specified time points), and follow-up neurodevelopmental outcome data were retrieved from the original records. In the CoolCap Study, infants were randomised at a median of 4.8 (IQR 4.1 to 5.3) h after birth. For the purposes of our analyses, time points refer to time elapsed after randomisation.

Statistical Analysis

Descriptive statistics were used to examine demographic, obstetric, and neonatal parameters in the entire cohort and then stratified by 12-h glycaemic profile (hypoglycaemia, hyperglycaemia, and normoglycaemia). Differences among the three groups were assessed with chi-square or Freeman-Halton tests for categorical variables; and ANOVA, Kruskal-Wallis, or multi-sample median (Brown-Mood) tests for continuous variables. Multivariable log-binomial regression models were used to generate adjusted risk ratios (aRR) and 95% CI for the treatment effect (i.e., impact of hypothermia therapy on the risk of an unfavourable outcome at 18 months). Each model was adjusted for Sarnat stage and 5-minute Apgar score. An interaction term between treatment type (hypothermia *vs.* standard therapy) and 12-h glycaemic profile was then added to determine whether the latter modified the treatment effect observed in the main effects model. In addition to relative measures of association, multivariable binomial regression was used to estimate adjusted risk differences and the number needed to treat (NNT). Since hypothermia could have influenced glycaemic profiles, we performed a sensitivity analysis that involved re-running all models using glycaemic profiles at 0-h (prior to beginning therapeutic hypothermia) as the potential effect modifier. All hypothesis tests were two-sided, and statistical significance for the final models was assessed at the 0.05 level. All analyses were performed using SAS 9.4 (SAS Institute, Cary, NC).

RESULTS

Early glycaemic profile and baseline characteristics

During the first 12 h, 9% (18/194) of infants had ≥1 episode of hypoglycaemia, 45% (87/194) had ≥1 episode of hyperglycaemia, and 46% (89/194) were normoglycaemic. Demographic and perinatal data stratified by 12-h glycaemic profiles are shown in table 1. Mothers of hyperglycaemic infants had higher rates of sentinel delivery complications (i.e., prolapsed cord, umbilical cord tear, placental abruption, and ruptured uterus) and emergency caesarean deliveries compared to normoglycaemic and hypoglycaemic infants. The distribution of glycaemic profile among therapeutic hypothermia and control infants was not statistically different (p=0.12, data not shown).

Table 1 Baseline infant and maternal characteristics according to glycaemic profile

Characteristics n (%) unless otherwise stated	All subjects (n=194)	Normoglycaemia (n=89)	Hypoglycaemia (n=18)	Hyperglycaemia (n=87)	p Value[a]
Infant					
Birth weight, mean (SD), g	3,470 (651)	3,438 (658)	3,416 (658)	3,513 (648)	0.81
Gestational age, mean **(**SD), wks	39.1 (1.5)	39.0 (1.5)	38.8 (1.8)	39.3 **(**1.5)	0.35
Male gender, n (%)	102 (53)	38 (43)	13 (72)	51 (59)	0.02
White race, n (%)	126 (65)	61 (69)	9 (50)	56 (64)	0.57
Apgar 5-min, median (IQR)	2.0 (0-4.0)	2.5 (0-3.0)	2.0 (0-4.0)	2.0 (0-3.0)	0.80
Apgar 5-min >3, n (%)	48 (25)	21 (24)	8 (44)	19 (22)	0.14
pH <1 hour of life, mean (SD)	6.87 (0.23)	6.89 (0.23)	6.80 (0.18)	6.87 (0.24)	0.35
Sarnat stage 3 at randomisation, n (%)	70 (36)	28 (32)	8 (44)	34 (39)	0.42
Hypothermia treatment, n (%)	99 (51)	47 (53)	5 (28)	47 (54)	0.12
Maternal					
Age, mean **(**SD), years	28.4 (6)	28.7 (6)	25.7 (7)	28.7 (7)	0.15
First child, n (%)	102 (53)	55 (62)	11 (61)	36 (41)	0.03
Gestational diabetes, n (%)	11 (6)	6 (7)	1 (6)	4 (5)	0.90
Preeclampsia/eclampsia, n (%)	14 (7)	7 (8)	1 (6)	6 (7)	0.99
Antibiotic therapy/pyrexia, n (%)	39 (20)	21 (24)	3 (17)	15 (17)	0.56
Emergency caesarean delivery, n (%)	132 (68)	50 (56)	12 (67)	70 (81)	0.002
Sentinel delivery complications, n (%)[b]	69 (36)	26 (29)	4 (22)	39 (45)	0.05
Other perinatal complications, n (%)[c]	42 (22)	23 (26)	2 (11)	17 (20)	0.34

[a]p Value from chi-square or Freeman-Halton tests for categorical variables, and ANOVA, Kruskal-Wallis, or multi-sample median (Brown-Mood) tests for continuous variables.

[b]Prolapsed cord, umbilical cord tear, placental abruption, and ruptured uterus.

[c]Umbilical cord knot, shoulder dystocia, feto-maternal bleeding, antepartum haemorrhage, traumatic instrument delivery, and head entrapment.

Univariate analysis of multi-organ function by glycaemic profile

Acid-base balance

Infants with hypoglycaemia during the first 12 h after randomisation were more acidotic at the time of randomization and at 12 h than normoglycaemic and hyperglycaemic infants (table 2), with significantly lower pH and bicarbonate levels, and larger base deficits. These parameters were not significantly different between hyperglycaemic and normoglycaemic infants.

Table 2 Univariate analysis of metabolic and clinical parameters by glycaemic profile

Metabolic/clinical parameter	Normoglycaemia (n=89)		Hypoglycaemia (n=18)		Hyperglycaemia (n=87)		p Value[a]		
	n	Mean (SD), Median (IQR), or n (%)	n	Mean (SD), Median (IQR), or n (%)	n	Mean (SD), Median (IQR), or n (%)	Hypo *vs.* Norm	Hyper *vs.* Norm	Hypo *vs.* Hyper
Acid-Base									
pH at 0 h	87	7.36 (0.13)	17	7.23 (0.16)	83	7.34 (0.10)	<0.001	0.11	0.005
pH at 12 h	84	7.41 (0.07)	15	7.30 (0.13)	82	7.39 (0.08)	0.002	0.22	0.01
HCO_3 at 0 h (mEq/L)	77	19.4 (7.7)	15	13.0 (5.6)	83	17.8 (6.6)	<0.001	0.08	0.005
HCO_3 at 12 h (mEq/L)	79	22.0 (4.8)	13	18.4 (5.0)	79	21.7 (5.2)	0.02	0.42	0.07
Base deficit at 0 h (mEq/L)	86	-5.5 (6.3)	18	-11.1 (9.1)	86	-6.3 (6.4)	0.005	0.15	0.02
Base deficit at 12 h (mEq/L)	84	-1.8 (4.3)	16	-7.0 (7.5)	83	-2.6 (4.2)	0.006	0.15	0.03
Liver and coagulation parameters									
AST at 0 h (IU/L)	76	275 (388)	14	878 (801)	79	364 (797)	0.003	0.61	0.004
AST at 24 h (IU/L)	73	318 (516)	9	2101 (2450)	76	394 (663)	0.001	0.22	0.002
ALT at 0 h (IU/L)	82	115 (239)	17	341 (376)	79	157 (420)	0.002	0.46	0.006
ALT at 24 h (IU/L)	79	159 (333)	11	531 (608)	77	136 (233)	0.01	0.97	0.009
PT at 0 h (sec)	80	18.2 (15.4-25.7)	14	26.1 (21.6-50.5)	75	20.1 (15.6-24.4)	0.02	0.19	0.02
PT at 24 h (sec)	78	15.4 (13.3-17.6)	10	26.4 (21.0-29.6)	72	15.6 (14.1-19.3)	0.05	0.74	0.04
PTT at 0 h (sec)	82	45.2 (36.4-62.6)	14	79.5 (44.0-93.2)	78	47.0 (37.0-60.8)	0.08	0.53	0.25
PTT at 24 h (sec)	79	39.0 (34.4-46.0)	9	44.3 (41.7-53.0)	75	43.0 (33.5-55.9)	0.01	0.20	0.73
Renal function									
Creatinine at 0 h (mg/dL)	81	1.2 (0.3)	16	1.5 (0.5)	80	1.2 (0.3)	0.02	0.29	0.002
Creatinine at 24 h (mg/dL)	79	1.3 (0.5)	13	1.9 (0.6)	80	1.3 (0.5)	<0.001	0.86	<0.001
Urine output 24 h (ml/kg/hr)	86	1.7 (1.3)	15	0.6 (0.6)	87	1.5 (1.3)	<0.001	0.31	0.001
Haematologic parameters									
Haematocrit at 0 h (%)	88	47.2 (8.2)	18	48.3 (8.0)	81	46.3 (8.7)	0.64	0.36	0.28
Haematocrit at 24 h (%)	80	47.3 (8.1)	12	45.2 (9.8)	78	46.9 (6.8)	0.64	0.95	0.74
Platelets at 0 h (10^9/L)	83	217 (71)	18	161 (81)	78	204 (81)	0.005	0.33	0.04
Platelets at 24 h (10^9/L)	76	200 (71)	12	130 (50)	78	175 (78)	0.007	0.09	0.12

Respiratory function									
PaO_2 at 0 h (mm Hg)	87	113 (74)	18	103 (107)	87	124 (95)	0.02	0.56	0.13
PaO_2 at 12 h (mm Hg)	84	91 (58)	16	74 (46)	85	87 (36)	0.09	0.87	0.06
$PaCO_2$ at 0 h (mm Hg)	89	33 (14)	18	31 (10)	87	32 (12)	0.84	0.97	0.98
$PaCO_2$ at 12 h (mm Hg)	86	35 (8)	16	36 (9)	85	36 (10)	0.74	0.67	0.89
FiO_2 at 0 h (%)	86	51 (29)	16	6 (33)	85	58 (31)	0.21	0.11	0.66
FiO_2 at 12 h (%)	83	37 (25)	13	58 (35)	83	39 (26)	0.05	0.21	0.12
Cardiovascular function									
Heart rate, mean through 12 h	88	125 (21)	18	144 (22)	87	133 (27)	0.002	0.07	0.08
Heart rate, mean through 24 h	88	125 (21)	18	144 (22)	87	133 (26)	0.002	0.06	0.08
Systolic BP, mean through 12 h	88	61 (9)	17	59 (14)	87	65 (10)	0.39	0.01	0.05
Systolic BP, mean through 24 h	88	61 (8)	17	59 (14)	87	65 (9)	0.44	0.01	0.10
Diastolic BP, mean through 12 h	88	41 (7)	17	38 (13)	87	44 (8)	0.09	0.02	0.01
Diastolic BP, mean through 24 h	88	41 (7)	17	38 (12)	87	44 (7)	0.14	0.01	0.02
MAP, mean through 12 h	88	50 (7)	17	46 (13)	87	53 (8)	0.15	0.02	0.03
MAP, mean through 24 h	88	49 (7)	17	46 (13)	87	52 (7)	0.18	0.03	0.03
Volume replacement, first 24 h, n (%)	89	52 (59)	18	12 (67)	87	46 (53)	0.60	0.54	0.31
Vasopressor given, first 24 h, n (%)	89	46 (52)	18	13 (72)	87	56 (64)	0.13	0.10	0.60
Central nervous system function									
EEG background at 0 h	89		18		87		0.33	0.82	0.66
Best, n (%)		4 (5)		2 (11)		6 (7)			
Intermediate, n (%)		56 (63)		9 (50)		53 (61)			
Worst, n (%)		29 (33)		7 (39)		28 (32)			
EEG seizure at 0 h, n (%)	89	57 (64)	18	10 (56)	87	55 (63)	0.60	0.99	0.60
EEG based prognosis group[16]	89		18		87		0.25	0.77	0.63
Best, n (%)		4 (5)		2 (11)		6 (7)			
Intermediate, n (%)		67 (75)		11 (61)		62 (71)			
Worst, n (%)		18 (20)		5 (28)		19 (22)			
Sarnat at 0 h	89		18		87		0.29	0.34	0.79
2 (moderate), n (%)		61 (69)		10 (56)		53 (61)			
3 (severe), n (%)		28 (32)		8 (44)		34 (39)			
Sarnat at 76 h	75		11		76		0.03	0.02	0.29

0/1 (mild), n (%)		18 (24)		4 (36)		15 (20)			
2 (moderate), n (%)		42 (56)		2 (18)		30 (40)			
3 (severe), n (%)		15 (20)		5 (46)		31 (41)			
Death or Sarnat stage 3 by 76 h, n (%)	78	18 (23)	16	10 (63)	78	33 (42)	0.005	0.02	0.17
HUS intracerebral haemorrhage, n (%)	83	2 (2)	15	1 (7)	82	3 (4)	0.40	0.68	0.50
Anticonvulsant therapy, n (%)	70	65 (93)	16	14 (88)	74	71 (96)	0.61	0.49	0.21
Primary outcome									
Died or severe disability at 18 months, n (%)	89	44 (49)	18	15 (83)	87	59 (68)	0.009	0.01	0.26

[a]p Value from chi-square or Freeman-Halton tests for categorical variables, and ANOVA, Kruskal-Wallis, or multi-sample median (Brown-Mood) tests for continuous variables.

HCO_3, bicarbonate (blood gas); AST, aspartate aminotransferase; ALT, alanine aminotransferase; PT, prothrombin time; PTT, partial thromboplastin time; PaO_2, partial pressure of oxygen; $PaCO_2$, partial pressure of carbon dioxide; FiO_2, fraction of inspired oxygen; BP, blood pressure; MAP, mean arterial pressure; EEG, electroencephalogram; HUS, head ultrasound.

Liver function

Liver enzymes were frequently elevated at randomisation and trended higher during the 24 h after randomisation. Aspartate aminotransferase (AST) and alanine transaminase (ALT) levels were significantly higher in hypoglycaemic infants compared to normoglycaemic and hyperglycaemic infants. AST and ALT levels were not significantly different between hyperglycaemic and normoglycaemic infants. Prothrombin time (PT) was significantly higher among hypoglycaemic infants; partial thromboplastin time (PTT) showed similar although mostly non-significant differences.

Renal function

Creatinine levels were frequently elevated at randomisation and increased during the next 24 h; levels were significantly higher among hypoglycaemic infants. Urine output (ml/kg/h) was significantly lower in hypoglycaemic infants during the first 24 h compared to the other glycaemic groups.

Haematologic parameters

Platelet counts were significantly lower among hypoglycaemic infants compared to normoglycaemic and hyperglycaemic infants, but were not significantly different between hyperglycaemic and normoglycaemic infants.

Cardiorespiratory function

Heart rate was highest among the hypoglycaemic infants, whereas hyperglycaemic infants had significantly higher mean arterial blood pressure during the first 24 h. There were no statistically significant differences between the glycaemic groups for partial pressure of oxygen and carbon dioxide.

Central nervous system function

Amplitude-integrated electroencephalogram (aEEG) parameters, Sarnat stage at randomisation, cranial ultrasound abnormalities, and the percentage of infants who received anticonvulsants were not significantly different between the glycaemic groups.

Primary outcome

The rate of death and/or severe neurological disability at 18 months (the primary outcome) was higher for hypoglycaemic (83%) and hyperglycaemic (68%) infants, compared to normoglycaemic (49%) infants.

Treatment effect by glycaemic profile

In the entire cohort, the rate of death or severe neurological disability at 18 months was lower among infants who were cooled (55%) compared to those who received standard therapy (67%) (table 3). After adjusting for Sarnat stage at randomisation and 5-min Apgar score, hypothermia therapy conferred a 23% relative risk reduction (aRR 0.77, 95% CI 0.63 to 0.94). After observing significant effect modification of hypothermia treatment, we investigated the treatment effect separately within each of the 12-h glycaemic profile groups. Only among hyperglycaemic infants did hypothermia therapy confer a statistically significant lower risk of the primary adverse outcome (aRR 0.80, 95% CI 0.66 to 0.99), with a 22% absolute risk reduction, and one case of death or severe disability at 18 months was averted for about every five hyperglycaemic infants who were cooled (NNT = 5). There were no statistically significant reductions in risk of the unfavourable primary study outcome associated with hypothermia therapy among normoglycaemic (aRR 0.95, 95% CI 0.70 to 1.27) or hypoglycaemic infants (aRR 1.03, 95% CI 0.52 to 2.00). Sensitivity analysis for differential treatment response of hypothermia in infants with glycaemic profiles based on the 0 h glucose values alone were similar, with statistically significant risk reduction only in hyperglycaemic infants (aRR 0.76, 0.58 to 0.99). Infants with

normoglycaemia (aRR 0.85, 0.61 to 1.18) and hypoglycaemia (aRR 0.92, 0.32 to 2.67) at 0 h did not significantly benefit from hypothermia treatment.

Table 3 Effect of hypothermia therapy on risk of death and/or severe neurological disability at 18 months

	Rate of primary outcome		Relative difference[a] in risk		Absolute difference[a] in risk		NNT[b]
Group	Cooled n/total (%)	Not cooled n/total (%)	Unadjusted RR (95% CI)	aRR (95% CI)	Unadjusted RD (95% CI)	aRD (95% CI)	
Overall	54/99 (55)	64/95 (67)	0.81 (0.64 to 1.02)	0.77 (0.63 to 0.94)	-13% (-26 to 1)	-16% (-28 to -3)	7
12-h Glucose profile							
Normoglycaemia	23/47 (49)	21/42 (50)	0.98 (0.64 to 1.49)	0.95 (0.70 to 1.27)	-1% (-22 to 20)	-5% (-26 to 16)	100
Hypoglycaemia	4/5 (80)	11/13 (85)	0.95 (0.58 to 1.55)	1.03 (0.52 to 2.00)	-5% (45 to 36)	1% (-53 to 55)	n/a
Hyperglycaemia	27/47 (58)	32/40 (80)	0.72 (0.54 to 0.96)	0.80 (0.66 to 0.99)	-23% (-41 to -4)	-22% (-39 to -4)	5

[a]The reference group consists of infants that are "not cooled" for all relative risk and risk difference models.

[b]The NNT is calculated from the adjusted risk difference point estimate and presents the number of infants that would have to be cooled in order to prevent 1 unfavourable 18-month outcome. The NNT is rounded to the next highest whole integer. The NNT is not estimated for the hypoglycaemia group since the point estimate does not reflect a risk reduction conferred by hypothermia therapy.

DISCUSSION

In this post hoc analysis of the CoolCap Study, we observed that early postnatal glycaemic profile (during the first 12 h after randomisation) was associated both with greater risk of deranged multi-organ function and with the response to induced hypothermia in infants with moderate-to-severe HIE. We have previously shown in the CoolCap study that the rate of death or severe neurological disability at 18 months age was lowest in normoglycaemic infants, intermediate in hyperglycaemic infants, and highest in hypoglycaemic infants.[15] Consistent with this pattern, in the current study we observed that multi-organ dysfunction, as defined by deranged laboratory results and clinical status was most severe in hypoglycaemic infants, intermediate in hyperglycaemic infants, and lowest in normoglycaemic infants. For the first time, this study shows that hyperglycaemic infants from the CoolCap Study benefitted significantly from hypothermia treatment, whereas hypoglycaemic and normoglycaemic infants showed no apparent benefit.

Therapeutic hypothermia and proposed adjuvant agents such as erythropoietin, xenon, melatonin have distinct neurotrophic, anti-apoptotic, anti-inflammatory, and anti-oxidant mechanisms that may contribute to their role in neuroprotection.[19-23] We speculate that individualised treatment with hypothermia plus specific adjuvant therapies directed to distinct pathophysiologic categories of infants with HIE may allow more optimal treatment than hypothermia alone.

Previous clinical studies have reported associations between deranged glucose homoeostasis and outcomes in infants with HIE.[11, 12, 18, 24-26] While we proposed a rather simplistic hypothesis that the severity, chronicity, and temporal pattern of perinatal hypoxia-ischaemia influences postnatal glycaemic profile, multi-organ dysfunction, and the response to hypothermia treatment,

it is important to appreciate that the aetiology and pathogenesis of deranged glucose homoeostasis in the setting of HIE are likely to be multifactorial, and its role as a biomarker of the underlying process or as a direct contributor to neuronal injury is not clearly understood.

Potentially, early hypoglycaemia may represent a category of HIE in which infants have depleted fetal energy reserves and decreased gluconeogenesis secondary to liver dysfunction. This could be related for example to more prolonged exposure to fetal hypoxia-ischaemia. Consistent with this sub-hypothesis, watershed–predominant lesions are more common in infants with HIE who have severe hypoglycaemia, and this pattern of injury has been associated with prolonged partial asphyxia.[27-29] Perhaps, many infants with moderate-to-severe HIE with severe multi-organ dysfunction (identified by early hypoglycaemia) are beyond the temporal therapeutic window or too severely injured to benefit from therapeutic hypothermia.

Early hyperglycaemia may represent another category of HIE, in which infants have milder multi-organ injury with intact gluconeogenesis and stress hormone responses, but have decreased glucose utilisation due to selective brain injury from perinatal asphyxia.[30, 31] In view of the retrospective nature of this analysis it is not possible to assess the impact, if any, of clinical glucose management; however, hyperglycaemia may occur during standard newborn glucose infusion rates. Speculatively, it is possible that the fetal hypoxic-ischaemic insult in at least some hyperglycaemic infants was temporally acute but relatively intense, consistent with the finding that this group had the highest rate of sentinel complications and emergency caesarean delivery. This would suggest that the evolution of brain injury in some hyperglycaemic infants may have been more likely to be within the therapeutic window for hypothermia to be beneficial.

Normoglycaemic infants, by contrast, had the least multi-organ dysfunction, had the lowest proportion of Sarnat stage 3 HIE at randomisation, and had the best outcomes. It is unclear why

this most stable group, with the best outcomes did not seem to benefit from therapeutic hypothermia. Analysis of other research cohorts in a meta-analysis is essential to confirm this finding.

Hypothermia itself may influence the glycaemic profile as noted by higher glucose levels at 4–24 h after randomisation in cooled infants in the original CoolCap Study cohort.[16] We therefore investigated the association of glycaemic profiles at 0 h (before hypothermia) with treatment response, and found similar and statistically significant benefits of hypothermia for hyperglycaemic infants only. This reinforces that early glycaemic profiles may represent distinct groups of infants with HIE, and are not confounded by treatment with hypothermia.

Some limitations of the present post hoc analysis should be considered. We do not have glucose values between the pre-specified time points or continuous values. The study, however, is strengthened by the determination of glucose and other metabolic and clinical parameters at pre-defined, discrete time points that were independent of clinical decisions. Glucose infusion rates, treatment thresholds, and interventions used were not standardised across centres, and variations in practice may have influenced the results. We also do not have information on the source (arterial, venous, or capillary) of all blood samples, laboratory test protocols, and monitoring devices, which may affect interpretation of our findings. Assessment of subsequent brain injury was limited because continuous EEG and MRI were not systematically done at all the centres. Further, there were few hypoglycaemic infants and many of them did not receive hypothermia, and thus this study had limited power to assess the impact of hypothermia on this subgroup.

CONCLUSIONS

Distinct clinical categories may be identified among infants with moderate-to-severe HIE based on the early postnatal glycaemic profile. These glycaemic profiles were associated with risk of multi-organ injury and with response to hypothermia treatment. Hypoglycaemic infants had the most severe multi-organ dysfunction, whereas hyperglycaemic infants had relative sparing of systemic organs. Critically, infants with hyperglycaemia appeared to benefit most from therapeutic hypothermia. Prospective studies are essential to investigate the role of early glycaemic patterns and their potential role in predicting response to therapeutic hypothermia, whether these patterns could guide individualised treatment protocols, and finally whether controlled euglycaemia could further improve outcomes in infants with moderate-to-severe HIE.

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The original study was designed by and was the responsibility of the Scientific Advisory Committee (SAC), who had full access to the trial data, and after reading and editing this manuscript, approved the final draft for submission.

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Contributors

SKB: conceptualized and designed the study, drafted the initial manuscript, and approved the final manuscript as submitted. JLS: performed data analysis, summarized results, critically reviewed the manuscript, and approved the final manuscript as submitted. AJG and JRK: conceptualized and designed the study, supervised data analysis and interpretation, reviewed and revised the manuscript, and approved the final manuscript as submitted. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Figure legends

Figure 1 Flow of infants through the trial.

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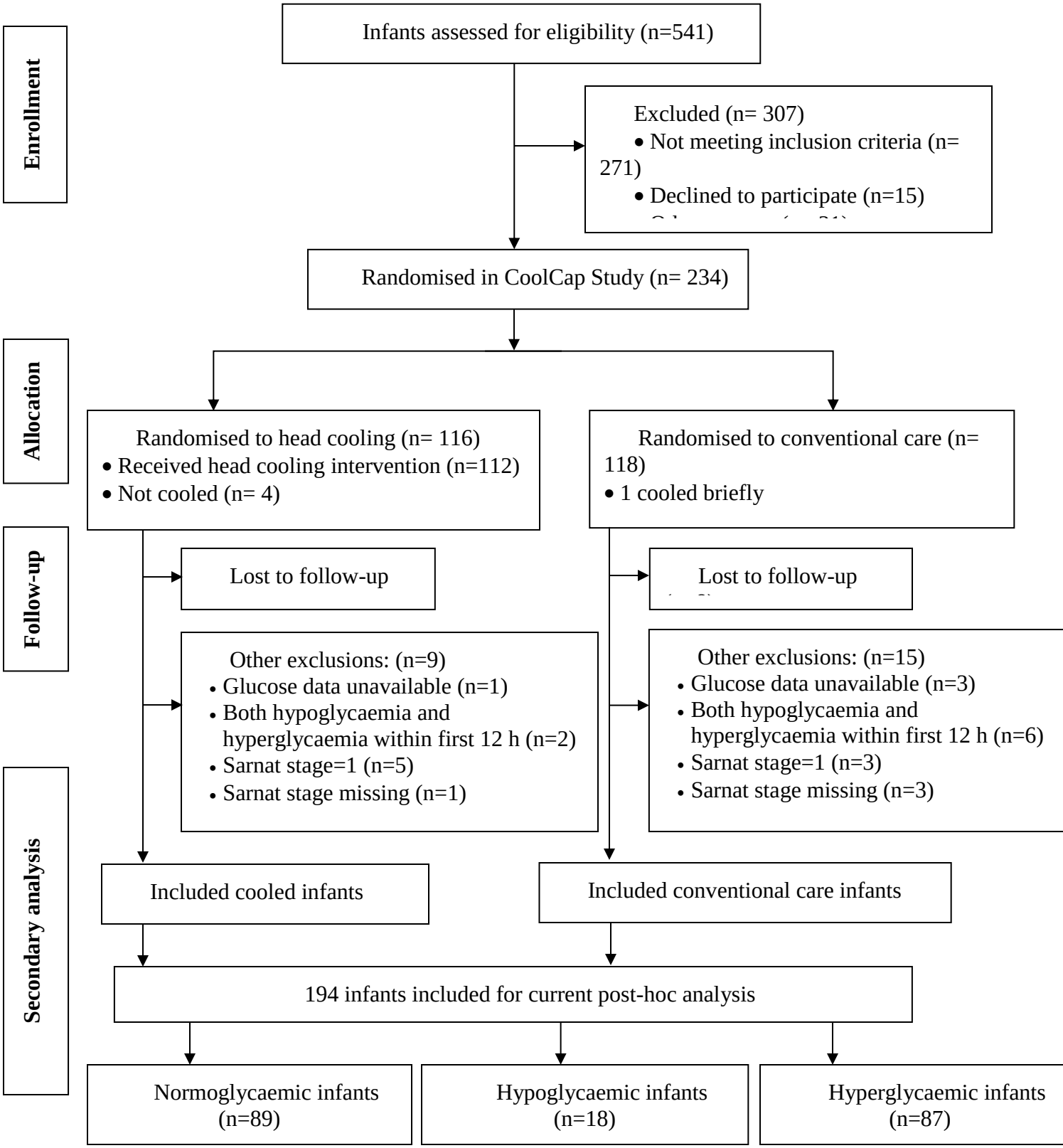
Enrollment
Infants assessed for eligibility (n=541)
Excluded (n= 307)
• Not meeting inclusion criteria (n= 271)
• Declined to participate (n=15)
Randomised in CoolCap Study (n= 234)
Allocation
Randomised to head cooling (n= 116)
• Received head cooling intervention (n=112)
• Not cooled (n= 4)
Randomised to conventional care (n= 118)
• 1 cooled briefly
Follow-up
Lost to follow-up
Lost to follow-up
Other exclusions: (n=9)
• Glucose data unavailable (n=1)
• Both hypoglycaemia and hyperglycaemia within first 12 h (n=2)
• Sarnat stage=1 (n=5)
• Sarnat stage missing (n=1)
Other exclusions: (n=15)
• Glucose data unavailable (n=3)
• Both hypoglycaemia and hyperglycaemia within first 12 h (n=6)
• Sarnat stage=1 (n=3)
• Sarnat stage missing (n=3)
Secondary analysis
Included cooled infants
Included conventional care infants
194 infants included for current post-hoc analysis
Normoglycaemic infants (n=89)
Hypoglycaemic infants (n=18)
Hyperglycaemic infants (n=87)